Cation Concentrations and Acidity in Breeding Ponds of the Spotted Salamander, *Ambystoma maculatum* (Shaw) (Amphibia: Ambystomatidae), in Virginia

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ABSTRACT.— For 8 years beginning in 1983, we monitored breeding activity by spotted salamanders, *Ambystoma maculatum*, in temporary ponds in eastern Virginia. In the spring of 1988, 1989, and 1990, a majority of the ponds examined (67.4%; N = 218) never contained egg masses or spermatophores. During the 8 years the number of egg masses declined severely in many ponds that were used as breeding sites. Ponds with breeding salamanders had significantly higher pH values than those that lacked breeding activity for all years, but were similar in pH to those with failed reproduction. Analysis of the 20 major cations indicated that successful ponds with large numbers of spotted salamander egg masses had lower aluminum, copper, and lead levels than ponds with declining populations of salamanders. Silicon levels were significantly higher in successful ponds. Stepwise discriminant function analyses indicate that high aluminum, copper, silicon, and zinc concentrations in breeding ponds are associated significantly with the decline in reproductive activity of spotted salamanders. The combination of elements in ponds maintaining stable or increasing populations of breeding spotted salamanders was distinctive; 91.8% were classified correctly as successful or failed ponds by canonical correlation analyses. Proximity of roads was not correlated with the concentration of any cation.

In general, it appears that low pH may produce mortality in amphibian eggs, larvae, and perhaps adults both by acid toxicity and by causing an increase in the concentration of free ions of toxic elements such as aluminum in the water column (Freda and Dunson 1985a). Acidity interferes with the ability of larvae to regulate internal concentrations of sodium, chloride, and perhaps other ions (Freda and Dunson 1985b). Additionally, low pH levels affect composition of the perivitelline fluid and the color and texture of the egg mass (Robb and Toews 1987). At extremely low pH (e.g. < 4.0) the egg capsule (perivitelline space) may shrink dramatically, killing the embryos (Pough 1976, Freda and Dunson 1985a, Blem and Blem 1989). In the spotted salamander, *Ambystoma maculatum* (Shaw), 50% or more of embryos die at pH 5.0 to 6.0 (Tome and Pough 1982) and all die at pH 4.0 or less (Pough and Wilson 1977, Cook 1983). However, in some ponds with relatively low

pH, substantial numbers of spotted salamanders may breed successfully (e.g. Cook 1983), suggesting that simple acidification is not the entire cause of mortality. Furthermore, high concentrations of organic materials in such ponds may ameliorate the impact of toxic ions by binding with them (Seip et al. 1984).

The present study documents concentrations of 20 of the more common cations present in the waters of temporary ponds used as breeding sites by spotted salamanders in Virginia and analyzes the relationships of water chemistry to reproductive success of the spotted salamander in these ponds.

MATERIALS AND METHODS

We counted egg masses in 218 temporary ponds in a 16-county area around Richmond, Va., in the spring (March-May) of 1988, 1989, and 1990. At least two counts per year were made in each pond, at least one of which was made well after all breeding activity ceased (usually late March). We have monitored pH and the number of egg masses in a majority of these ponds for 8 years (see Blem and Blem 1989). In addition, we measured the pH of each pond two to four times each year.

In March 1989 we obtained a 20-ml water sample from each of 48 temporary ponds that had contained egg masses over most of the 8 years of the study. The surface area, depth, and pH of each pond were measured at the same time. All 48 ponds were similar in altitude, exposure, and surrounding vegetation. All were in forests consisting of loblolly pine (*Pinus taeda*) or mixed pine-hardwoods. The two most widely separated ponds were 64 km apart. The ponds seldom froze during late spring and only ponds of sufficient depth to protect eggs from freezing (i. e. > 20 cm deep) were studied. Egg masses of spotted salamanders were counted in each pond, and pH was measured with an Orion SA250 portable pH meter. The water samples were filtered through No. 4 filter paper and analyzed for tannic acid using the HACH (1975) test. Samples of filtered water were stored in cleaned 35-ml borosilicate EPA water analysis vials, and were analyzed by the Chemical Analysis Laboratory at the Institute of Ecology, The University of Georgia, by means of inductively coupled argon plasma analysis. Twenty cations were quantified (Table 1). Replicate samples were run from several ponds to test for sampling variation; no determination differed by more than 8%. All data sets were tested to determine if they were normally distributed (SAS 1985; UNIVARIATE), and those deviating from normality were transformed to natural logarithms before further analyses were performed (Zar 1984).

We categorized ponds as "successful" (> 20 egg masses were present and produced larvae in all 8 years and the number did not

Table 1. Chemistry of water samples taken from spotted salamander breeding ponds in March, 1989.[a]

Variable	Successful ponds (N = 25)		Failed ponds (N = 23)		All ponds (N = 48)	
Al	0.44 ±	0.04	0.91 ±	0.15[b]	0.66 ±	0.08
B	1.48 ±	0.07	1.55 ±	0.08	1.51 ±	0.0
Ba	0.20 ±	0.01	0.22 ±	0.01	0.21 ±	0.01
Ca	5.27 ±	0.62	5.77 ±	0.51	5.51 ±	0.40
Cd	0.001 ±	0.0004	0.001 ±	0.0004	0.001 ±	0.0002
Cr	0.002 ±	0.0006	0.002 ±	0.0004	0.002 ±	0.0004
Cu	0.010 ±	0.008	0.025 ±	0.003[b]	0.018 ±	0.004
Fe	0.53 ±	0.12	1.06 ±	0.35	0.78 ±	0.18
K	4.84 ±	1.70	2.35 ±	0.54	3.65 ±	0.93
Mg	1.30 ±	0.20	1.33 ±	0.12	1.31 ±	0.12
Mn	0.08 ±	0.02	0.12 ±	0.03	0.10 ±	0.02
Na	14.46 ±	1.21	17.07 ±	2.34	15.71 ±	1.20
Ni	0.003 ±	0.001	0.007 ±	0.002	0.005 ±	0.001
P	0.065 ±	0.016	0.050 ±	0.016	0.058 ±	0.010
Pb	0.005 ±	0.003	0.013 ±	0.002[b]	0.009 ±	0.002
Si	6.557 ±	0.93	4.490 ±	0.40[b]	5.568 ±	0.54
Sr	0.027 ±	0.003	0.030 ±	0.003	0.028 ±	0.002
Zn	0.028 ±	0.004	0.026 ±	0.002	0.027 ±	0.002
pH	5.34 ±	0.15	5.29 ±	0.14	5.32 ±	0.10
Tannic acid	6.3 ±	0.8	6.8 ±	0.9	6.6 ±	0.8
Depth (cm)	34.4 ±	3.2	37.8 ±	3.3	36.1 ±	2.3
Area (m^2)	683.6 ±	201.1	632.6 ±	138.5	658.1 ±	170.0

[a] All ion concentrations represent total values (soluble + bound). All values for elements are means ± SE ppm; cobalt and molybdenum concentrations were below the level of detection.

[b] Failed ponds significantly different from successful ones, MANOVA tests of logarithmically transformed data, $P < 0.05$.

decline by more than 10% during the study) or "failed" (the number of egg masses deposited has declined by 80% or more and/or all egg masses failed to produce viable larvae throughout the study). We compared the physical characteristics and the levels of each element in successful ponds with those in failed ponds by means of multiple analysis of variance (SAS Institute 1985, MANOVA). Canonical correlation analyses (SAS Institute 1985, CANCORR) were used to determine whether successful ponds were different in overall element composition from those that failed. Stepwise discriminant function analyses (SAS Institute 1985, STEPDISC) were used to identify the elements that were correlated with success or failure of ponds. A 5% significance level ($P < 0.05$) was used in all tests, unless noted otherwise.

Table 2. Correlation coefficients for pH and elements in water from breeding ponds of the spotted salamander. All data not normally distributed were transformed logarithmically.

	Al	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Si	Sr	Zn
B	-.09																	
Ba	.18	.45ᵃ																
Ca	-.02	.23	.56ᵃ															
Cd	-.10	-.03	-.16	.02														
Cr	-.03	.10	.04	.11	-.05													
Cu	-.07	.26	.35ᵃ	.40ᵃ	-.09	.33ᵃ												
Fe	.61ᵃ	.10	.31ᵃ	.04	.06	.02	-.07											
K	.03	.08	.13	.08	.09	.33ᵃ	.39ᵃ	-.15										
Mg	-.17	.04	.39ᵃ	.75ᵃ	.08	.14	.32ᵃ	-.04	.11									
Mn	.05	.19	.36ᵃ	.23	.17	-.03	.46ᵃ	.20	.14	.41ᵃ								
Na	-.18	.27	.36ᵃ	.52ᵃ	-.05	.08	.42ᵃ	-.11	.14	.40ᵃ	.22							
Ni	.32ᵃ	-.03	.19	.04	-.05	.09	.15	.35ᵃ	-.13	.25	.25	-.07						
P	-.06	-.04	.20	.17	.16	.32ᵃ	.09	.17	.30ᵃ	.31ᵃ	.22	-.14	-.01					
Pb	-.30ᵃ	-.04	.01	-.07	.10	-.14	.04	-.33ᵃ	.45ᵃ	-.01	-.22	.10	-.02	.02				
Si	-.01	.04	.05	.16	-.01	.22	.22	.05	.38ᵃ	.26	-.19	.01	-.10	.14	.17			
Sr	-.13	.09	.53ᵃ	.89ᵃ	-.02	.03	.28ᵃ	.06	.02	.86ᵃ	.12	.55ᵃ	-.10	.17	.01	.19		
Zn	.02	.30ᵃ	.59ᵃ	.48ᵃ	-.07	.32ᵃ	.75ᵃ	.14	.38ᵃ	.53ᵃ	.53ᵃ	.40ᵃ	.25	.29ᵃ	.06	.08	.37ᵃ	
pH	-.08	.02	.002	.55ᵃ	-.03	.18	-.06	.16	.02	.38ᵃ	-.20	.17	-.21	.16	-.18	.36ᵃ	.42ᵃ	-.12

[a] $P < 0.05$.

RESULTS

Only 71 of 218 potential breeding ponds that we checked in 1988, 1989, and 1990 contained egg masses. The incidence of egg laying by spotted salamanders in those ponds declined between 1988 and 1990 by 23.9% (649 egg masses in 1988, 494 masses in 1989, 450 masses in 1990). By 1990, nearly 75% of all egg masses were in only 20 of the original 71 study ponds. The pH of the 71 ponds was 5.64 ± 0.07 SE in 1988, 5.30 ± 0.08 in 1989, and 5.54 ± 0.08 in 1990. The pH of ponds with no evidence of salamander breeding activity during the 3-year period was significantly lower in all 3 years (5.32 ± 0.07, 5.01 ± 0.06, and 5.12 ± 0.08, respectively). Acidity varied from year to year, depending upon the amount and possibly the pH of rainfall; acidity increased as ponds dried up in late spring.

In the 48 ponds in which we studied water chemistry (25 successful, 23 failed), no significant difference was detected in the pH of successful versus failed ponds (Table 1). Of the 20 elements measured, 18 were found at detectable levels. Cobalt and molybdenum were absent or were at concentrations below detectable levels. Aluminum, copper, and lead were at significantly higher concentrations in failed ponds than in successful ones (Table 1). Silicon concentrations were significantly higher in successful ponds. There was no statistical difference in pond depth or in pond surface area between successful and failed ponds (Table 1). Because some ponds were near roads, we were concerned about the effects of vehicular emissions and of chemical treatment of highways on element levels. Comparison by t-tests of the 16 ponds near roads (100 m or less) with 32 remote ponds did not reveal any significant difference in pH or in concentration of an element.
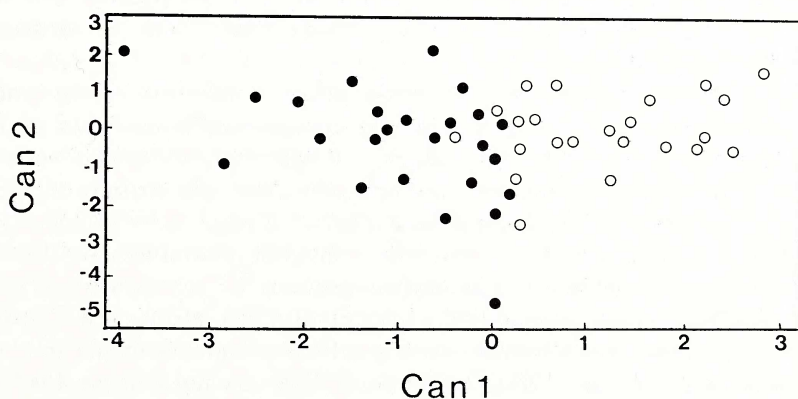

Fig. 1.   Canonical correlation analyses of the effect of cations in successful breeding ponds (closed circles) and failed ponds (open circles).

Pearson correlation coefficients (Table 2) indicate that only calcium concentration was correlated significantly with pH ($P < 0.001$). A number of other elements were correlated positively with each other and many of these were metals. For example, zinc and copper, calcium and magnesium, strontium and magnesium, and strontium and calcium have correlation coefficients > 0.7. Some of these elements apparently influence one another's abundance. Canonical correlation analyses (Fig. 1) indicate that successful ponds are distinctive; 91.8% of the ponds were identified correctly by the analyses. Major loading was on aluminum and sodium (first canonical correlation) and copper and silicon (second canonical correlation). Aluminum, copper, silicon, and zinc are the only significant cations in stepwise discriminant function analyses of successful and failed ponds (Table 3).

DISCUSSION

In the 1970s and early 1980s, the spotted salamander was abundant locally in the lower piedmont and coastal plain of Virginia, a zone of relatively high acid deposition from precipitation (Schwartz 1989). Recently we observed severe declines in the number of egg masses of the spotted salamander at some sites (Blem and Blem 1989). For example, one site had more than 500 egg masses in 1984, but the number declined annually, and in 1990 only 15 masses were deposited. At the same time the pH of this pond decreased from > 5.0 to < 4.0. During the same period we detected a general decrease in survivorship of spotted salamander eggs and embryos throughout eastern Virginia (Blem and Blem 1989). In general, there has been a trend for only a few ponds with pH values between 6.0 and 7.0 to support salamanders. However, the increased mortality has not been uniform. Some acidic ponds continue to support substantial numbers of egg masses, which have not declined during that period.

A correlation between decreased pH of temporary ponds and decreased survival of spotted salamander eggs and larvae has been recognized for more than a decade (e.g. Pough 1976). Mortality seems to increase at a pH below 6.0, although substantial proportions of eggs survive to hatch at pH values from 4.0 to 6.0 (Pough 1976, Pough and Wilson 1977, Saber and Dunson 1978, Ling et al. 1986, Blem and Blem 1989). Complete lethality to embryos appears to be in the range of 3.5 to 4.5 (Pierce 1985, Freda 1986, Ling et al. 1986, Robb and Toews 1987), although this depends upon levels of certain elements in the breeding pond (Pough 1981, Dale et al. 1985, Freda and Dunson 1985a, 1986). Recently it was found that mortality may be associated with concentrations of specific elements such as aluminum (Albers and Prouty 1987, Clark and Hall 1985, Clark and LaZerte 1985, Freda and

Table 3. Stepwise discriminant function analyses of the importance of elements in the use of breeding ponds by spotted salamanders.

Element	Partial R^2	Wilks' Lambda[a]
Aluminum	0.209	0.808
Copper	0.232	0.650
Silicon	0.240	0.589
Zinc	0.161	0.494

[a]Elements and associated statistics are listed in order of entry in the analyses. All Wilks' Lambda values are significant at $P < 0.05$.

Dunson 1985a), calcium (Freda and Dunson 1985a), magnesium (Freda and Dunson 1985a), and sodium (Freda and Dunson 1984, 1985a). However, there seem to be few data regarding the ambient levels of cations in temporary ponds, and little information regarding the combined effect of a more complex suite of ions (see Gascon and Planas 1986).

The chemistry of temporary waters on the lower piedmont and coastal plain of Virginia is complex. The chemical composition of the water in these ponds, along with its physical characteristics and local biotic influences, may all vary between ponds even when they are separated by only short distances. Year-to-year variations in water depth, temperature, and duration are important determinants of reproductive success and continued use of ponds by spotted salamanders (see Shoop 1974, Albers and Prouty 1987). The surrounding forest and soil are important influences on levels of biologically significant cations (James and Riha 1986). Coastal plain and lower piedmont soils of Virginia vary widely in composition (Blem and Blem 1989), and it is not surprising that elements detected in temporary ponds are also extremely variable. For example, several of our test ponds had aluminum concentrations of 2-3 ppm; 0.3 ppm is considered extremely high in Virginia waters (C. Lunsford, personal communication). Furthermore, the relationship between pH and aluminum toxicity appears to be complex (Freda et al. 1989). For example, organically bound aluminum may be harmless (Freda 1986) or less harmful (Seip et al. 1984) than free aluminum ions; therefore ponds with large amounts of humic acids could have high concentrations of aluminum without harm to aquatic life. Also, aluminum may ameliorate acid toxicity at intermediate concentrations and low pH values (Freda et al. 1989).

Only traces of copper were found in many ponds, but others had levels as high as 0.179 ppm. Copper is known to be toxic to amphibians

(National Academy of Sciences 1974), although the degree of toxicity has been determined for only a few species. Likewise, lead levels of some ponds were in the range of concentrations known to be detrimental (0.75-20 μg/liter; USEPA 1980a). On the other hand, zinc levels of only 79 ppm were obtained and levels of 180-540 μg/liter are considered to be detrimental to aquatic organisms (USEPA 1980b). The relationship between silicon levels and successful use of breeding ponds is puzzling. Silicon concentrations in successful ponds were significantly higher than in those that failed (Table 1). We do not know of any relationship between silicon and viability of aquatic life.

Some elements have complex effects on success of hatching of amphibian eggs, e.g. a few elements appear to be harmful at both high and low concentrations. For example, at a pH of 4.25, hatching and survival of embryos of the Jefferson salamander, *Ambystoma jeffersonianum* (Green), are greatest at magnesium concentrations of about 20 mg/liter, but the hatching rate declines at both lower and higher concentrations (Freda and Dunson 1985a). The effects of most other elements increase with decreased pH, but the degree of synergism with other elements is unknown. It appears that failed ponds in Virginia have distinctive cation compostions (Fig. 1). Stepwise discriminant function analyses suggest that aluminum, copper, silicon, and zinc all may play a role in mortality of spotted salamander larvae. Failed and successful ponds did not differ in pH. Rather, it appears that at the present levels of acidity, ponds become fatal because of their elemental composition.

The few surveys of the chemistry of temporary ponds used for breeding by amphibians have considered either restricted suites of chemical elements or have included relatively few ponds (e.g. Dale et al. 1985, Freda and Dunson 1986, Albers and Prouty 1987). Conclusions about the importance of specific chemicals in temporary ponds have been mixed. For example, Freda and Dunson (1986) concluded that mortality may be influenced by significant interactions of pond pH and other chemical variables, whereas Albers and Prouty (1987) found that pond longevity, water temperature, and oxygen content were more significant in spotted salamander reproduction than chemical alterations brought about by acid precipitation. Those findings are not incompatible, given the range of study areas, times, and substances considered.

LITERATURE CITED

Albers, P. H., and R. M. Prouty. 1987. Survival of spotted salamander eggs in temporary woodland ponds of coastal Maryland. Environ. Pollution 46:45-61.

Blem, C. R., and L. B. Blem. 1989. Tolerance of acidity in a Virginia population of the spotted salamander, *Ambystoma maculatum* (Amphibia: Ambystomatidae). Brimleyana 15:37-45.

Clark, K. L., and R. J. Hall. 1985. Effects of elevated hydrogen ion and aluminum concentrations on the survival of amphibian embryos and larvae. Can. J. Zool. 63:116-123.

Clark, K. L., and B. D. LaZerte. 1985. A laboratory study of the effects of aluminum and pH on amphibian eggs and tadpoles. Can. J. Fish. Aquat. Sci. 42:1544-1551.

Cook, R. P. 1983. Effects of acid precipitation on embryonic mortality of *Ambystoma* salamanders in the Connecticut Valley of Massachusetts. Biol. Conserv. 27:77-88.

Dale, J. M., B. Freedman, and J. Kerekes. 1985. Acidity and associated water chemistry of amphibian habitats in Nova Scotia. Can. J. Zool. 63:97-105.

Freda, J. 1986. The influence of acidic pond water on amphibians: a review. Water, Air, Soil Pollut. 30:439-450.

Freda, J., V. Cavdek, and D. G. McDonald. 1989. Role of organic complexation in the toxicity of aluminum to *Rana pipiens* embryos and *Bufo americanus* tadpoles. Can. J. Fish. Aquat. Sci. 47:217-224.

Freda, J., and W. A. Dunson. 1984. Sodium balance of amphibian larvae exposed to low environmental pH. Physiol. Zool. 57:435-443.

Freda, J., and W. A. Dunson. 1985a. The influence of external cation concentration on the hatching of amphibian embryos in water of low pH. Can. J. Zool. 63:2649-2656.

Freda, J., and W. A. Dunson. 1985b. Field and laboratory studies of ion balance and growth rates of ranid tadpoles chronically exposed to low pH. Copeia 1985:415-423.

Freda, J., and W. A. Dunson. 1986. Effect of low pH and other chemical variables on the local distribution of amphibians. Copeia 1986:454-466.

Gascon, C., and D. Planas. 1986. Spring pond water chemistry and the reproduction of the wood frog, *Rana sylvatica*. Can. J. Zool. 64:543-550.

HACH. 1975. HACH Water and Wastewater Analysis Procedures Manual. 3rd ed. HACH Chemical Co., Ames, Ia.

James, B. R., and S. J. Riha. 1986. pH buffering in forest soil organic horizons: relevance to acid precipitation. J. Environ. Qual. 15:229-234.

Ling, R. W., J. P. VanAmberg, and J. K. Werner. 1986. Pond acidity and its relationship to larval development of *Ambystoma maculatum* and *Rana sylvatica* in upper Michigan. J. Herpetol. 20:230-236.

National Academy of Sciences. 1974. Amphibians: guidelines for the breeding, care, and management of laboratory animals. Washington, D.C.

Pierce, B. A. 1985. Acid tolerance in amphibians. BioScience 35:239-243.

Pough, F. H. 1976. Acid precipitation and embryonic mortality of spotted salamanders, *Ambystoma maculatum*. Science 192:68-70.

Pough, F. H. 1981. Mechanisms by which acid precipitation produces embryonic death in aquatic vertebrates. U.S. Dept. Interior Office Res. Tech., Res. Proj. Tech. Compl. Rep., 1-10.

Pough, F. H., and R. E. Wilson. 1977. Acid precipitation and reproductive success of *Ambystoma* salamanders. Water, Air, Soil Pollut. 7:307-316.

Robb, L., and D. Toews. 1987. Effects of low ambient pH on perivitelline fluid of *Ambystoma maculatum* (Shaw) eggs. Environ. Pollut. 44:101-107.

Saber, P. A., and W. A. Dunson. 1978. Toxicity of bog water to embryonic and larval anuran amphibians. J. Exp. Biol. 204:33-42.

SAS Institute, Inc. 1985. SAS User's Guide: Statistics. Version 5 edition. Cary, N.C.

Schwartz, S. E. 1989. Acid deposition: unraveling a regional phenomenon. Science 243:753-762.

Seip, H. M., L. Muller, and A. Naas. 1984. Aluminum speciation: comparison of two spectrophotometric analytical methods and observed concentrations in some acidic aquatic systems in southern Norway. Water Air Soil Pollut. 23:81-95.

Shoop, R. C. 1974. Yearly variation in larval survival of *Ambystoma maculatum*. Ecology 55:440-444.

Tome, M. A., and F. H. Pough. 1982. Responses of amphibians to acid precipitation. Acid rain/fisheries. Pages 245-254 *in* Proceedings of an International Symposium on Acid Precipitation Fishery Impacts in Northeastern North America. Am. Fish. Soc., Bethesda, Md.

U.S. Environmental Protection Agency. 1980a. Ambient Water Quality Criteria for Lead. EPA 440/5-80-057. Natl. Tech. Inf. Serv., Springfield, Va.

U.S. Environmental Protection Agency. 1980b. Ambient Water Quality Criteria for Zinc. EPA 440/5-80-079. Natl. Tech. Inf. Serv., Springfield, Va.

Zar, J. H. 1984. Biostatistical Analysis. Prentice-Hall, Inc. Englewood Cliffs, N.J.